

Materials List for:

Identification of Intracellular Signaling Events Induced in Viable Cells by Interaction with Neighboring Cells Undergoing Apoptotic Cell Death

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Materials

Name	Company	Catalog Number	Comments
Cell culture materials			
DMEM, 1x with [4.5 g/L] glucose, L-glutamine & sodium-pyruvate	Mediatech (Manassas, VA)	10-013-CV	
HyClone Fetal Clone II, fetal bovine serum	GE Healthcare (Logan, UT)	SH30066.03	add 10% (v/v) to the culture medium A
Penicillin-Streptomycin-Glutamine, 100x	Life Technologies (Grand Island, NY)	10378-016	add 100 units/mL to the culture media A and B
γ-Interferon, mouse recombinant, from <i>Escherichia coli</i>	Calbiochem, Millipore (Billerica, MA)	407303	add 10 units/mL to the culture medium A
Falcon polystyrene tissue-culture treated dishes, non-pyrogenic	Corning (Durham, NC)	353003	sterile, 100 mm diameter
Falcon polystyrene centrifuge tubes, conical bottom	Corning (Durham, NC)	352095	sterile, 15 mL
Trypsin-EDTA, 0.05%, Gibco	Fisher (Waltham, MA)	25300062	sterile, 100 mL
Name	Company	Catalog	Comments
Chemicals and inhibitors			
Staurosporine, from <i>Streptomyces sp.</i>	Sigma (St. Louis, MO)	S4400	use at [1 µg/mL] for 3 h to induce apoptosis
Epidermal growth factor (EGF), [1 mg/mL]	Millipore (Billerica, MA)	EA140	use at 50 nM to stimulate responder cells
UltraPure, 0.5 M EDTA, pH 8.0	Life Technologies (Grand Island, NY)	15575-038	use 5 mM to detach adherent cells
Trypan blue solution, 0.4%	MP Biomedicals (Santa Ana, CA)	91691049	for dead cells staining
Paraformaldehyde Solution, 4% in DPBS	Affymetrix (Santa Clara, CA)	19943	use 0.4% (v/v) in 1x DPBS for cell fixation
Dulbecco's phosphate-buffered saline (DPBS), 1x, Gibco	Life Technologies (Grand Island, NY)	14040133	stock solution
Dulbecco's phosphate-buffered saline (DPBS), Ca ²⁺ - and Mg ²⁺ -free, 1x, Gibco	Life Technologies (Grand Island, NY)	14190367	stock solution
Cytochalasin D, 4 mM in dimethyl sulfoxide (DMSO), from <i>Zygosporium mansonii</i>	Sigma (St. Louis, MO)	C8273	use at [4 µg/mL] to inhibit phagocytosis
Dimethyl sulfoxide (DMSO)	Sigma (St. Louis, MO)	D2650	solvent for cytochalasin D

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Cell lysis buffer ingredients			
Tris-buffered saline (TBS), 10x, pH 7.4	BioRad (Hercules, CA)	1706435	for cell lysis buffer
Sodium pyrophosphate	Sigma-Aldrich (St. Louis, MO)	221368	10 mM for cell lysis buffer
Sodium deoxycholate	Sigma-Aldrich (St. Louis, MO)	D6750	0.5% w/v for cell lysis buffer
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich (St. Louis, MO)	L3771	0.1% w/v for cell lysis buffer
Glycerol	Sigma-Aldrich (St. Louis, MO)	G5516	10% for cell lysis buffer
Sodium fluoride	Sigma-Aldrich (St. Louis, MO)	450022	25 mM for cell lysis buffer
Complete mini EDTA-free protease inhibitor cocktail tablet	Roche Diagnostics (Indianapolis, IN)	4693159001	for cell lysis buffer, 1 tablet per 10 mL of buffer
Triton X-100	Sigma-Aldrich (St. Louis, MO)	T8787	10% for cell lysis buffer
Dithiothreitol (DTT)	MP Biomedicals (Santa Ana, CA)	11DTT00005	1 mM for cell lysis buffer
Phenylmethanesulfonyl fluoride (PMSF)	MP Biomedicals (Santa Ana, CA)	4800263	1 mM for cell lysis buffer
Sodium orthovanadate	MP Biomedicals (Santa Ana, CA)	2159664	10 mM for cell lysis buffer
Name	Company	Catalog	Comments
Equipment			
Sorvall T6000B centrifuge	Du Pont (Wilmington, DE)	T6000B	
Fisher Tissuemat, water bath	Fisher (Waltham, MA)		
Sonic Dismembrator (sonicator), model 100	Fisher (Waltham, MA)		
Miscellaneous			
Cell counting chamber, Neubauer	Hawksley (Lancing, Sussex, UK)	AC2000	
Annexin V-FITC Apoptosis Detection Kit II	Millipore (Billerica, MA)	CBA059	for PI and Annexin V staining to determine the stage of apoptosis
Eppendorf microcentrifuge tubes, 1.5 mL	Sigma-Aldrich (St. Louis, MO)	T9661	
Transwell permeable support, 0.4 μ m polycarbonate membrane	Corning (Corning, NY)	3413	sterile, polystyrene, tissue culture treated
Name	Company	Catalog	Comments
Antibodies			
Phospho-glycogen synthase kinase-3 α / β (GSK-3 α / β) (Ser21/9), polyclonal antibody	Cell Signaling Technology	9331	rabbit polyclonal antibody
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibody ((14C10))	Cell Signaling Technology	2118	rabbit monoclonal antibody